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Comparative evaluation of vacuum-based surface sampling methods for collection of *Bacillus* spores

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Abstract

In this study, four commonly-used sampling devices (vacuum socks, 37 mm 0.8 μm mixed cellulose ester (MCE) filter cassettes, 37 mm 0.3 μm polytetrafluoroethylene (PTFE) filter cassettes, and 3MTM forensic filters) were comparatively evaluated for their ability to recover surface-associated spores. Aerosolized spores ($\sim 10^5$ CFU cm^{-2}) of a *Bacillus anthracis* surrogate were allowed to settle onto three material types (concrete, carpet, and upholstery). Ten replicate samples were collected using each vacuum method, from each material type. Stainless steel surfaces, inoculated simultaneously with test materials, were sampled with pre-moistened wipes. Wipe recoveries were utilized to normalize vacuum-based recoveries across trials. Recovery (CFU cm^{-2}) and relative recovery (vacuum recovery/wipe recovery) were determined for each method and material type. Recoveries and relative recoveries ranged from 3.8×10^3 to 7.4×10^4 CFU cm^{-2} and 0.035 to 1.242, respectively. ANOVA results indicated that the 37 mm MCE method exhibited higher relative recoveries than the other methods when used for sampling concrete or upholstery. While the vacuum sock resulted in the highest relative recoveries on carpet, no statistically significant difference was detected. The results of this study may be used to guide selection of sampling approaches following biological contamination incidents.

Keywords

Surface sampling; Anthrax; *Bacillus anthracis*; Bioterrorism agent; Vacuum sampling

1. Introduction

Environmental sampling for pathogens (bacteria, viruses, fungi, etc.) can be a critical component in some disease outbreak investigations. Assessing the surface contamination

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provides valuable information to epidemiologists regarding the source and potential number of people exposed to the contaminant. Surface sampling methods are also used to determine the spatial extent and magnitude of the contaminant. These data are necessary for planning the decontamination strategy, and subsequently assessing the efficacy of decontamination treatment for clearance decisions (Gilchrist, 1992; Schulster et al., 2012; Emanuel et al., 2008). During the investigation and remediation that followed the 2001 *Bacillus anthracis*-contaminated letter attacks, about 120,000 environmental samples were collected by law enforcement, public health officials, United States (US) Environmental Protection Agency responders, US Postal Service and contract personnel (U.S. Government Accountability Office, 2005), and processed by many laboratories across the country (Canter, 2005). At the time, little was known about the efficiency of the sampling devices and methods used in collecting and recovering *B. anthracis* spores from surfaces, and no standard laboratory processing methods were available. In 2005, a Government Accountability Office (GAO) report expressed concern that validated sampling and detection methods were needed (U.S. Government Accountability Office, 2005). Since then, standardized sampling procedures and validated processing methods for detecting *B. anthracis* from smooth, non-porous surfaces have been developed (Hodges et al., 2010; Rose et al., 2011; CDC, 2012). Use of these methods in future investigations will enable more effective interpretation of sampling results leading to better response decisions related to remediation and public health. These two methods, however, are best suited to sampling smooth, nonporous surfaces and are limited in the size of area that can be sampled yet still retain their collection efficiency. If another widespread *B. anthracis* contamination event were to occur, responders would need additional methods for sampling large surface areas, as well as the ability to sample porous surfaces such as carpet, upholstery, and concrete. Vacuum-based methods are thought to be superior for sampling both rough and porous surfaces, however, few vacuum devices are available that are appropriate for the collection of samples for microbial analysis targeting *B. anthracis* spores. Further, not all vacuum methods currently in use have been characterized for their recovery efficiency of bacterial spores.

Brown et al., investigated the efficiency of vacuum socks when used to recover *Bacillus atrophaeus* spores from carpet, wallboard and steel (Brown et al., 2007b), and Estill et al. (2009) compared the vacuum socks to wipes as tools to recover *B. anthracis* spores from carpet. Another collection device, the 37 mm filter cassette (loaded with MCE or PTFE filters) has been evaluated for sampling aerosolized spores (Burton et al., 2005), and for the collection of metal dust from surfaces (ASTM, 2005), but not for the collection of bacterial spores from surfaces. The 3M™ Forensic Filter (3M, St. Paul, MN) is intended for use in crime investigations for the collection of fibers, hairs, and other types of evidence. Frawley et al. (2008) evaluated the Forensic Filters for recovery of *B. anthracis* spores from surfaces as compared to swabs and wipes. In each of the studies mentioned above, one vacuum-based method was evaluated using a spore deposition method, surface area, and processing method unique to that study, making comparisons across devices and studies difficult. In order to properly compare the collection efficiency of multiple methods, they should be evaluated under one test design that utilizes inoculation methods, spore preparations, test material surfaces, and collection procedures that are consistent throughout.

The goal of this current study was to compare four vacuum devices for their relative abilities to recover *B. atrophaeus* spores (surrogate for *B. anthracis*) from three different porous surface types. One device (vacuum sock) was evaluated at two sampling speeds (slow and fast), resulting in a total of five vacuum methods being compared.

2. Materials and methods

2.1. Preparation of material coupons

Stainless steel (16-gauge, 304 or 316 stainless; Dillon Supply, Raleigh, NC) was cut into 35.6 cm by 35.6 cm coupons from larger pieces of stock material (Fig. 1A). Carpet, concrete, and upholstery coupons were utilized as representative indoor and outdoor surfaces (Fig. 1B–E). Carpet coupons were prepared by affixing one 30.5 cm by 30.5 cm carpet tile (Home Depot, Model 3W05300100, pile height 0.64 cm) to a 35.6 cm by 35.6 cm by 1.9 cm piece of plywood, using the self-adhesive backing plus three staples on each side. Concrete coupons were prepared by mixing Sakrete® Sand/Topping Mix (Atlanta, GA) according to the manufacturer's instructions and pouring into 35.6 cm by 35.6 cm by 3.81 cm molds. Surfaces were smoothed by a hand trowel and allowed to cure for at least 5 days under plastic sheeting. Prior to use in trials, loose grit was removed from the concrete surfaces by spraying them with water using a gas-powered pressure washer. Upholstery coupons were prepared by placing a 30.5 cm by 30.5 cm piece of upholstery padding (432 Poly Foam, Online Fabric Store, Item# 1243310) on top of a 35.6 cm by 35.6 cm by 1.9 cm piece of plywood, then covering with a 61 cm by 61 cm piece of fabric (Indoor/Outdoor Modern Houndstooth Red Fabric, www.fabric.com, Part# UJ-849). The fabric was pulled tight over the foam surface and the excess fabric was secured by staples on the rear side of the plywood.

Stainless steel and concrete coupons were placed inside sterilization bags (Item# 62020TW, General Econopak, Inc., Philadelphia, PA), and sterilized by subjecting them to a one hour gravity autoclave cycle at 121 °C and 103 kPa. Carpet and upholstery coupons were sterilized by 240 min exposure to 400 parts per million by volume (ppmv) H₂O₂ generated by a STERIS VHP® ED 1000 generator (STERIS Corp., Mentor, OH). Residual H₂O₂ was allowed to desorb from the coupons for a minimum of two days before the coupons were used in experiments. Prior to testing, coupon sterility was confirmed by swab sampling (~25 cm²) one coupon from each sterilization batch, streaking the swab onto tryptic soy agar plates (TSA; Difco, Franklin Lakes, NJ) and incubating the plates at 35 ± 2 °C for 18–24 h. If contamination was detected, the entire batch was resterilized.

2.2. Bacterial spore preparation and coupon inoculation

Spores of *B. atrophaeus* (ATCC 9372; formerly *Bacillus subtilis* var. *niger* and *Bacillus globigii*) (Nakamura, 1989) were used as surrogates for the biological agent *B. anthracis*. Spore preparations were obtained from the US Army Dugway Proving Ground (Utah), and have been described previously (Brown et al., 2007a). These spores were prepared specifically for use as a *B. anthracis* surrogate during surface sampling studies (Brown et al., 2007a). Coupon surfaces were inoculated via metered dose inhaler (MDI) according to the aerosol deposition-based inoculation methods described previously (Calfee et al., 2013; Lee

et al., 2011), yielding $\sim 10^5$ spores cm^{-2} over the 929 cm^2 coupon surface. A single MDI was utilized across the entire trial for each material type. Each inoculation event consisted of one actuation from the MDI. One stainless steel control coupon was inoculated at the beginning, middle, and end of the test material inoculation sequence and served as reference coupons. Pre-moistened gauze wipe-based sample recoveries from these coupons were used to confirm the magnitude and precision of the inoculation, across the entire set of dosed coupons.

2.3. Experimental design

Four vacuum-based sampling devices; vacuum sock, 37 mm mixed cellulose ester (MCE) filter cassette, 37 mm polytetrafluoroethylene (PTFE) filter cassette, and forensic evidence filter (Table 1) were evaluated for their ability to recover aerosol-deposited spores from various surfaces (carpet, concrete, and upholstery) (Figs. 1 and 2). The vacuum sock was evaluated using both a slow and a fast pace, resulting in a total of five methods being evaluated. Each of the five methods was evaluated in a series of five trials; in each trial one sampling method was tested on all three surface types (Table 2). Ten replicate samples were collected for each method and each material type. In addition, wipe sampling of stainless steel surfaces (reference coupons) was conducted in order to normalize recoveries across trials. Normalization was necessary since trials were conducted on numerous days, using different MDIs to inoculate the test coupons. Surfaces were sampled after the 18 hours allotted for spore deposition (gravitational settling in the chamber). Components of all wipe- and vacuum-based sampling devices were assembled aseptically into sampling kits prior to experimentation. For all sampling procedures, a two-person team was utilized and consisted of a sampler and a sample handler. Both members of the team donned a new pair of nitrile gloves (non-sterile) before collection of each sample. The sample handler supported the sampler by placing coupons, removing the aerosol deposition apparatus, recording data, operating the vacuum equipment, and handling the bagged samples. The sampler's sole responsibility was to handle the sterile template, and operate the sampling device to collect the sample. This strategy was employed to mimic field collection procedures and to reduce the potential for cross-contamination. The amount of surface area sampled by each method was also consistent with field-use procedures. Following sample collection, all samples were placed into primary and secondary containment for transport to the laboratory for analysis.

Wipe-based sampling of stainless steel control samples was accomplished using gauze wipes (Kendall Versalon™ 8042, Mansfield, MA) according to the methods described previously (CDC, 2012; Brown et al., 2007a; Busher et al., 2008). Wipes were pre-moistened with 2.5 mL of phosphate buffered saline with 0.05% Tween® 20 (PBST; Sigma Aldrich, St. Louis, MO) prior to use. For all coupons, the centermost 30.5 by 30.5 cm portion (the area inoculated) of the 35.6 by 35.6 cm coupon was delineated with a sterile template and subsequently sampled.

2.4. Vacuum sampling

Vacuum socks (Midwest Filtration, Cincinnati, OH) (Fig. 2A) were used to collect samples from surfaces according to procedures described previously (Brown et al., 2007b). Sterilized (gamma irradiated, 15 kGy) vacuum socks, packaged individually in vacuum-sealed bags

were purchased from the vendor. The center-most 30.5 cm by 30.5 cm portions of three inoculated coupons were sampled for each vacuum sock sample, for a total area sampled equal to 2787 cm². Consistent with field procedures, sterile templates were used to delineate this area during sampling. Vacuum sock samples were collected using either a “fast” or “slow” procedure. The sampler traversed the coupon surface at a pace of 1–2 or 3–5 s per linear foot when using the fast or slow procedure, respectively. Surfaces were sampled in a back-and-forth manner with about 50% overlap of each sweep. Each coupon was sampled first in the horizontal direction, then again in an orientation rotated 90° from the first. The vacuum equipment utilized with the vacuum socks was the OmegaVac (Atrix, Int.; Burnsville, MN), which drew airflow of about 2000 L min⁻¹ through the socks (Table 1). After sampling, the sock was removed from the cardboard tube holders, the opening secured with a zip-tie, and placed in a specimen cup for transport to the lab.

Two types of 37 mm filters were evaluated with the cassette-based vacuum devices; a 0.8 µm pore-size MCE and a 0.3 µm pore-size PTFE type (Fig. 2B, Table 1). The MCE and PTFE filters were evaluated using identical collection and processing procedures. For both, a 2.5 cm length of Tygon® tubing (0.635 cm inside diameter, SKC Int.; part# 225-1345) was affixed to the inlet side of the cassette with a polyvinyl chloride (PVC) adapter (SKC Int., 225-132A), and a 20 cm piece with PVC adapter was affixed to the outlet side of the cassette. The terminus of the inlet tube was cut at a 45° angle (Fig. 2B). A Vac-U-Go pump (SKC, Int.; part# 228-9605), with a flow rate calibrated to 20 L min⁻¹, was used to draw air through the device. For each sample, the center-most 30.5 cm by 30.5 cm portion of the inoculated coupon, delineated by a sterile template, was sampled by lightly pressing the angled tube to the coupon surface, and traversing the coupon at a rate of 3–5 s per linear foot. A total area of 929 cm² (1 coupon) was sampled for each of ten replicates per material type. After sample collection, the 2.5 cm Tygon® tube and associated PVC adaptor were removed from the cassette and placed into a sterile 15 mL conical tube. The 20 cm Tygon® tube was removed from the cassette, and manufacturer-supplied plastic plugs placed into both the inlet and outlet of the filter cassette in order to contain the sample.

Collection of samples with the forensic filter (3M, St. Paul, MN) proceeded similar to the vacuum socks, using a 3–5 s per linear foot traverse speed and a sampled area of 2787 cm² (3 coupons) for each of ten replicates per material type. An adjustable transformer (ISE, Inc.; part# 3PN1010B) was used to reduce the flow rate of the OmegaVac to ~790 L min⁻¹ to prevent sampling device malfunction (filter collapse) during use. Following sample collection, the forensic filter unit was removed from the vacuum hose, and the manufacturer-supplied plugs were affixed on both open ends to contain the sample.

2.5. Extraction and recovery

All samples were transported from the point of collection to the analysis laboratory within primary and secondary containment, and tertiary containment within plastic storage bins. The secondary containment and storage bins were decontaminated with Dispatch® sporicidal wipes (Clorox Corp., Oakland, CA) before transport. When received by the analysis laboratory, the bins, as well as each primary and secondary container, were again decontaminated with sporicidal wipes. To the extent possible, all extractive and analytical

methods were conducted within a Class II Biological Safety Cabinet (BSC), using aseptic technique.

Spores were extracted from wipe samples by first placing the wipe into a 50 mL conical tube, then adding 20 mL phosphate buffered saline with 0.05% Tween 20 (PBST) to each tube and agitating the tubes using a vortex mixer (set to maximum rotation) for 2 min in 10 s intervals. Spores were extracted from vacuum sock samples similarly to the methods described by Brown et al. (2007b). Briefly, the collection portion of the vacuum sock was wetted by dipping into a sterile 133 mL specimen cup (VWR, Radnor, PA; part# 25384-144) containing 20 mL sterile PBST, then cut into segments with sterile scissors. The cups, containing the segmented sock, were then agitated (30 min, 300 rpm) on a rotating laboratory shaker to dislodge collected spores from the sock material. To minimize contaminant handling, wipes and segmented socks remained in the extraction vessel after the extraction procedure.

Collected spores were extracted from the 37 mm cassette devices by first adding 5 mL PBST to the 15 mL conical tube containing the 2.5 cm Tygon® tube and PVC adapter. The tubes were then sonicated 1 min at 40 kHz in an ultrasonic water bath (Branson Model 8510; Danbury, CT) and subsequently agitated by vortexing for 2 min. The 5 mL eluant was then transferred to a 60 mL jar (Container & Packaging Supply, Eagle ID, part# J037, lid part# L208). With the cassette outlet plug in place, the inlet plug was removed, 1 mL of PTSB was pipetted into the orifice and the plug was returned. The cassette was then rotated such that the added liquid wetted all surfaces of the filter and cassette. Next, the filter cassette was opened using a specialized tool (SKC, Int.; part# 225-8372), with the cassette in the upright orientation so that no liquid was spilled. An additional 1–2 mL of PBST was then used to rinse the interior of the cassette, and all liquids inside the cassette were captured by pipette and transferred to the 60 mL jar. The filter was then aseptically removed from the cassette and placed into the 60 mL jar. With the filter removed, the remaining portion of the filter cassette was rinsed with an additional 3–4 mL of PBST, and transferred to the jar. For each extraction, a total volume of 11 mL PBST was used (5 mL for nozzle extraction, 6 mL for cassette rinse). The volume of extract recovered was determined using a 10 mL serological pipette (graduated to 12.5 mL). The filter and both liquid fractions, now combined in the 60 mL jar, were sonicated for 3 min to dislodge spores; the jars were rotated 90° within the water bath after 1.5 min. The fragmented filter remained inside the sample cup following the extraction procedure.

The forensic filter samples were extracted by first removing the device from the primary containment bag and wiping the plastic housing with a sporicidal wipe (Dispatch®, Clorox Corp.). With the filter in the upright position (collection side pointing up), the collection side plug was carefully removed and 5 mL from a 90 mL stock of PBST was dispensed into the device. The plug was returned to the device, and the device was rotated to wet all surfaces of the filter and housing. A sterile scalpel was then used to cut the red tamper-evident tape securing the two halves of the device. While oriented again with the collection side up, the two halves of the device were carefully separated and the nozzle side was placed to the side (with nozzle pointing downward) in a rack. Using sterile forceps, the filter was removed from the device and placed into a stomacher bag (part# BA6090; Seward, Inc., West Sussex,

UK). A 10 mL serological pipette was used to transfer any liquid remaining within the device to the bag. A sterile plastic 5 mL transfer pipette was used to dispense fresh PBST, from the stock into the bottom of the device housing, rinse the housing, and recollect the liquid. The same procedure was then repeated for the top portion of the housing. All wash liquid along with the remaining volume from the 90 mL PBST stock was added to the stomacher bag, and the bag was closed by folding according to the manufacturer's instructions. The bag was then placed inside a second stomacher bag for containment, and the second bag was closed. The dually contained filter and liquid were then placed inside a stomacher (Seward Model 400 Circulator, Seward, Inc.) and the sample was agitated at 260 rpm for 1 min. Foam generated during agitation was allowed to subside for 10 min. The liquid was collected and dispensed into two 50 mL conical tubes using a 50 mL serological pipette. The sample was then sedimented by centrifugation at $3500 \times g$ for 15 min (room temperature). All but about 3 mL of supernatant was then carefully removed from each tube by pipette, and the pellets were resuspended by vortexing 30 s followed by brief sonication. Lastly, the two 3 mL extracts for each sample were combined into one 50 mL tube.

For all sample types, extracts were diluted 10-fold in series (in PBST) and 0.1 mL spread-plated onto TSA in triplicate. Plates were incubated at $35 \pm 2^\circ\text{C}$ for 18–24 h and colony forming units (CFU) were enumerated visually. Only plates containing between 30 and 300 CFU were utilized for recovery estimates. Extracts were diluted and replated if none of the 10-fold dilutions resulted in all three plates containing colony counts within the acceptable range. All extracts were stored at $4 \pm 2^\circ\text{C}$.

2.6. Data treatment

Total spore recovery was calculated by multiplying the mean CFU counts from triplicate plates by the inverse of the volume plated (e.g., $1/0.1$ or 10), by the dilution factor, and finally by the volume of the extract. The mean total recoveries for each trial (method and material) were determined by averaging the mean of all sample replicates ($n = 10$ for vacuum samples, $n = 5$ for wipe samples).

To normalize spore recoveries across experiments, all recoveries obtained by vacuum methods were divided by the recoveries obtained by wipe sampling stainless steel positive control reference coupons collected on the same day. These wipe samples were collected from stainless steel surfaces that were inoculated simultaneously with test coupons. Normalization was conducted to reduce bias associated with comparing samples from different test days, which were inoculated with separate MDIs. Multiple MDIs were required (typically one per experiment) since each MDI provides only 200 doses. The resulting values, hereafter referred to as “relative recoveries”, were ratios of vacuum method recovery to wipe sample positive control recovery. These data were analyzed by one-way ANOVA for each material type. Bonferroni *post-hoc* tests were subsequently conducted to evaluate each contrast. Significance was assessed using a *p*-value equal to 0.05. SigmaPlot 11 (Systat Software Inc., San Jose, CA) was utilized for these abovementioned statistical analyses.

3. Results

Spore recovery from individual wipe samples used to sample stainless steel control coupons was between 3×10^4 and 2×10^5 CFU cm⁻² across all trials. The mean recoveries for wipe samples ranged from $4.2 \times 10^4 \pm 2.2 \times 10^4$ CFU cm⁻² to $1.2 \times 10^5 \pm 6.0 \times 10^4$ CFU cm⁻² across the trials (Table 3). Within trial variability (C_v) in spore recoveries from wipe samples was lower than between trial, as recovery never varied more than 52% for any one trial (C_v range = 21.1% to 52.0%).

The mean spore recoveries achieved by the vacuum-based sampling methods ranged from $3.8 \times 10^3 \pm 3.8 \times 10^3$ CFU cm⁻² (forensic filter, upholstery) to $7.4 \times 10^4 \pm 3.9 \times 10^4$ CFU cm⁻² (37 mm MCE, concrete) across the trials (Table 3, Figs. 3–5). Variability in recovery data for any one method and one material type was between 17.3% (forensic filter, carpet) and 100.6% (forensic filter, upholstery). The mean variability (averaged across all three materials for each method) was 31.2%, 28.9%, 40.5%, 56.1%, and 49.5% for the vacuum sock (slow), vacuum sock (fast), 37 mm (MCE), 37 mm (PTFE), and forensic filter methods, respectively.

Relative recoveries were between 0.035 (forensic filter, upholstery) and 1.24 (37 mm MCE, concrete) across the trials (Figs. 3–5). A relative recovery greater than 1.0 suggests that the vacuum method was more efficient at recovering surface-bound spores than the wipe-based method deployed on a stainless steel surface.

Comparisons (ANOVA) of the relative recoveries indicated that the 37 mm MCE method out-performed all other methods when used to sample concrete or upholstery (both $p < 0.001$) (Table 3). For carpet, the vacuum sock (slow) achieved the highest relative recovery (0.64). However, this relative recovery from carpet was not significantly higher than that of the 37 mm MCE method ($p = 0.22$). Vacuum sock (slow) recovery from carpet was significantly higher than the vacuum sock (fast) ($p = 0.008$), 37 mm (PTFE) ($p < 0.001$), and forensic filter ($p < 0.001$).

4. Discussion

Vacuum-based sampling was used extensively following the 2001 bioterror incident for the purposes of detection and characterization of surface-associated *B. anthracis* spores (Teshale et al., 2002). Nonetheless, significant gaps remain in our understanding of vacuum-based sampling methods (Piepel et al., 2012; U.S. Government Accountability Office, 2005). The current study was conducted to determine which of the few currently-used vacuum-based surface sampling methods are most efficient at *Bacillus* spore collection. Recoveries were determined for each vacuum method, using sample collection procedures as they would be deployed during an actual contamination incident (to the extent possible).

It has been demonstrated that inoculation method (liquid versus aerosol) can affect recoveries from surface sampling methods such as swabbing (Edmonds et al., 2009). It is expected that inoculation method can have an even larger impact on vacuum-based sampling recoveries, since such methods do not use direct surface contact and therefore are greatly affected by spore properties (e.g., spore coat, surface charge, and suspension materials) and

their interaction with surfaces. Accordingly, the current study utilized an aerosol-based inoculation method, as to more accurately represent previous bioterror surface contamination. The current MDI-based method was previously shown to generate particles in which the majority (~85%) contained one or two spores (Lee et al., 2011, Carrera et al., 2005).

Recoveries from wipe samples of stainless steel surfaces (controls) were within the expected range, as demonstrated previously (Calfee et al., 2013). Variability in positive control recoveries between trials ($C_v = 33.4\%$) and within trials (C_v between 21.1 and 52.0%) was similar to that reported when liquid inoculation was used to dose coupons (Buhr et al., 2011; Calfee et al., 2011; Rastogi et al., 2009; Wood et al., 2011). Nonetheless, since the maximum and minimum recovery values for individual positive controls among the trials varied by more than 100% (maximum value was more than twice the minimum), and unlike direct liquid inoculations, absolute values for the amount of spores deposited onto the coupons surfaces are difficult to determine; vacuum-based recovery data were normalized by control recoveries (wipe samples of stainless steel). This approach helped to reduce the inoculum as a source of variation, and allowed recoveries to be compared across trials. A more randomized test design was considered for text execution, in order to make direct comparisons across methods without normalization. However, the large number of test replicates generated in the current study required multiple trials over multiple test days. Accordingly, comparisons across test days, and therefore normalization, were necessary. The systematic test design aided in the ability to collect numerous samples on a single test day, as sampling personnel and personnel conducting the laboratory analyses were able to streamline methods through batch processing.

When visually comparing relative recoveries across the trials (Figs. 3–5), it is apparent that the vacuum sock (slow and/or fast) and the 37 mm (MCE) methods consistently recovered more spores than the other methods. ANOVA results indicated that the 37 mm MCE method achieved significantly higher relative recoveries than the other methods when concrete and upholstery were sampled. While ANOVA indicated significant differences in the methods with regard to recoveries from carpet, no single method was significantly better than all other methods when sampling from this material type. For instance, the vacuum sock (slow) method was superior to all methods with the exception of the 37 mm MCE filter cassette.

Previously, it was demonstrated that wipe-based surface sampling procedures recover about 39% of spores seeded onto stainless steel (Brown et al., 2007a). Although the sampling area was much larger in the current study (929 cm² versus 25 cm²), we can assume that spore recovery efficiencies in the current study were similar to those reported previously (Brown et al., 2007a). Using the recovery efficiency estimates reported by Brown et al. (2007a), the test inoculums in this study were between 1.0×10^5 and 3.1×10^5 spores per cm². Using these calculated trial-specific inoculums; one can gain a perspective of the vacuum-based method recovery efficiencies. While this treatment is similar to the normalization described herein, by accounting for the recovery inefficiency of the wipe (as reported in the literature) one can more directly compare to recovery efficiencies reported elsewhere. Vacuum sock (slow) recovery efficiencies were 25.1%, 10.2%, and 4.2% for carpet, concrete, and upholstery, respectively. These respective recovery efficiencies for the vacuum sock (fast)

were 15.2%, 11.7%, and 9.1%. These values are similar to recovery efficiencies reported previously for this method; 28.2% and 18.9% on carpet and concrete, respectively (Brown et al., 2007b). The highest calculated recovery efficiency (48.7%) was for the 37 mm MCE filter cassette, when used to sample concrete; the lowest (1.4%) was for the forensic filter when used to sample upholstery. Overall, the recovery efficiencies achieved by the forensic filter was one order of magnitude higher than those reported previously for this device (Frawley et al., 2008). These differences are likely due to the differences in inoculation methods (Edmonds et al., 2009). The current study utilized aerosol-deposited spores while the previous study utilized liquid inoculation.

A large portion of recovery inefficiency is presumably due to the inability to extract collected spores from the sample media (Da Silva et al., 2012). We speculate that both types of membrane filters used in 37 mm cassette device likely have fewer spores tightly bound to the collection media as compared to the matrices of the vacuum sock and forensic filters. Indeed, others have demonstrated excellent extraction efficiencies for spores collected on MCE and PTFE membrane filters (Clark Burton et al., 2005). This phenomenon may partially explain the superior recoveries of the 37 mm MCE method when used to sample concrete and upholstery.

Comparatively low recoveries were obtained by the forensic filter (Table 3, Figs. 3–5). Initially, the forensic filter was utilized in the same manner as the vacuum sock, using the OmegaVac without a transformer to reduce the airflow velocity supplied. It was immediately apparent that the pressure generated by the pump was incompatible with the device, as the filter and filter support frequently buckled under the amount of pressure drop generated, allowing airflow (and spores) to bypass the filter. To remedy the malfunction, a series of tests were conducted to determine the flow rate at which the filter media would not collapse. The determined flow rate was then utilized for all trials. One can speculate that the lowered flow rate necessitated by the device failures had a negative impact on spore recoveries achieved. Particle resuspension from the surface and capture by the device may have been lessened by the reduced air velocity, and therefore negatively affecting sampler efficiency. However, when the ratio of airflow (lpm) to device inlet cross-sectional area (mm^2) was calculated to gain a rudimentary understanding of air velocity at the point of particle aspiration; the 37 mm cassette devices had the lowest ratio (0.3, flow rate divided by orifice area) yet the highest recovery from several materials. Vacuum sock had the highest ratio (3.5) and the forensic filter had a ratio of 1.4. This analysis suggests that inlet flow velocity is not a good determinant of overall sampler efficiency.

One factor for consideration when selecting a vacuum-based sampling method is the amount of surface area sampled per unit of time. The diameter of the device inlet nozzle, to a large extent, determines the number of passes required to completely sample a particular area, and thus the amount of time required to collect the sample. The nozzle widths (inside diameter) of the vacuum sock, 37 mm cassette, and forensic filter devices are 27 mm, 9 mm, and 57 mm, respectively. Accordingly, sampling with the forensic filter takes about half the amount of time, per unit area, as the vacuum sock. The vacuum sock takes about one third the amount of time as the 37 mm cassette. For example, on one square foot of surface area, sampling with the vacuum sock (slow), vacuum sock (fast), 37 mm cassette (MCE or

PTFE), and forensic filter would each require about 60, 60, 100, and 30 total passes, and about 240, 90, 400, and 120 s, respectively. The vacuum sock and forensic filter devices offer clear advantages with regard to the number of samples collected per unit of effort (sampling time per unit area). Such an advantage may be desirable when collecting vast numbers of samples, such as during characterization sampling following a wide-area incident (Buttner et al., 2004; Franco and Bouri, 2010; Schmitt and Zacchia, 2012). It has been suggested that using robotic sampler to generate composite samples could decrease the amount of effort expended by both sample collection teams and analysis laboratories (Lee et al., 2013).

Another factor for consideration when selecting a sampling method is the burden the generated samples pose to the processing laboratory (Franco and Bouri, 2010). When samples were processed in batch sizes suitable to typical microbiology laboratories, the approximate amount of time required for processing the vacuum sock, 37 mm filters, and forensic filters was 7.5, 12, and 18 min per sample, respectively. These values include all extraction procedures required and exclude the time for dilution plating. These estimates assume that all consumables have been aliquoted, sterilized, and prepared in advance. Typical batch sizes for vacuum sock, 37 mm cassette, and forensic filters were 12, 10, and 10 samples, respectively. Our evaluations suggest that laboratory throughput would likely be highest for the vacuum sock samples. However, in a large-area incident, many labs will be needed to process and analyze samples in a timely manner. The capabilities of the available processing labs may need to be considered when deciding upon the preferred methods for sample collection.

Lastly, it should be noted that the coupons utilized in the current study were free of dirt, grime, and background organisms. Co-collection of such particles could affect (negatively or positively) collection efficiencies, and analytical methods (likely negatively). Further studies aimed at determining these effects are needed (Piepel et al., 2012).

5. Conclusions

Four vacuum-based sampling methods were comparatively evaluated for their ability to recover *Bacillus* spores from environmental surfaces. ANOVA results indicated that recoveries, when using the 37 mm MCE filter cassette method, were significantly higher for concrete and upholstery sampling when compared to the other methods. Recoveries were similar between the vacuum sock (slow) and 37 mm MCE method when sampling carpet. While collection and recovery efficiency are important factors for the selection of a preferred sampling method, other factors such as sample collection speed and ease of laboratory analysis methods should also be considered. The data reported here can be used to inform public health officials and incident responders regarding sampling methodologies during a biological contamination incident.

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References

- ASTM. D71440 — Standard Practices for Collection of Surface Dust by Microvacuuming Sampling for Subsequent Metals Determination. ASTM International; West Conshohocken, PA: 2005.
- Brown GS, Betty RG, Brockmann JE, Lucero DA, Souza CA, Walsh KS, Boucher RM, Tezak M, Wilson MC, Rudolph T. Evaluation of a wipe surface sample method for collection of *Bacillus* spores from nonporous surfaces. *Appl. Environ. Microbiol.* 2007a; 73:706–710. [PubMed: 17122390]
- Brown GS, Betty RG, Brockmann JE, Lucero DA, Souza CA, Walsh KS, Boucher RM, Tezak MS, Wilson MC. Evaluation of vacuum filter sock surface sample collection method for *Bacillus* spores from porous and non-porous surfaces. *J. Environ. Monit.* 2007b; 9:666–671. [PubMed: 17607386]
- Buhr TL, Young AA, Minter ZA, Wells CM, Shegogue DA. Decontamination of a hard surface contaminated with *Bacillus anthracis* Delta Sterne and *B. anthracis* Ames spores using electrochemically generated liquid-phase chlorine dioxide (eClO₂). *J. Appl. Microbiol.* 2011; 111:1057–1064. [PubMed: 21824240]
- Burton NC, Adhikari A, Grinshpun SA, Hornung R, Reponen T. The effect of filter material on bioaerosol collection of *Bacillus subtilis* spores used as a *Bacillus anthracis* simulant. *J. Environ. Monit.* 2005; 7:475–480. [PubMed: 15877169]
- Busher, A.; Noble-Wang, J.; Rose, L., editors. *Sampling for Biological Agents in the Environment*. ASM Press; Washington, DC: 2008.
- Buttner MP, Cruz P, Stetzenbach LD, Klima-Comba AK, Stevens VL, Emanuel PA. Evaluation of the Biological Sampling Kit (BiSKit) for large-area surface sampling. *Appl. Environ. Microbiol.* 2004; 70:7040–7045. [PubMed: 15574898]
- Calfee MW, Choi Y, Rogers J, Kelly T, Willenberg Z, Riggs K. Lab-scale assessment to support remediation of outdoor surfaces contaminated with *Bacillus anthracis* spores. *J. Bioterror. Biodefense.* 2011; 2:1–8.
- Calfee MW, Lee SD, Ryan SP. A rapid and repeatable method to deposit bioaerosols on material surfaces. *J. Microbiol. Methods.* 2013; 92:375–380. [PubMed: 23384827]
- Canter DA. Addressing residual risk issues at anthrax cleanups: how clean is safe? *J. Toxicol. Environ. Health.* 2005; 68:1017–1032.
- Carrera M, Kesavan J, Zandomeni R, Sagripanti JL. Method to determine the number of bacterial spores within aerosol particles. *Aerosol Sci. Technol.* 2005; 39:960–965.
- CDC. Emergency response resources [Online]. Atlanta, GA: 2012. Available: <http://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthraxis.html> [Accessed June 28, 2013]
- Clark Burton N, Adhikari A, Grinshpun SA, Hornung R, Reponen T. The effect of filter material on bioaerosol collection of *Bacillus subtilis* spores used as a *Bacillus anthracis* simulant. *J. Environ. Monit.* 2005; 7:475–480. [PubMed: 15877169]
- Da Silva SM, Urbas AA, Filliben JJ, Morrow JB. Recovery balance: a method for estimating losses in a *B. anthracis* spore sampling protocol. *J. Appl. Microbiol.* 2012; 114(3):807–818. [PubMed: 23210624]
- Edmonds JM, Collett PJ, Valdes ER, Skowronski EW, Pellar GJ, Emanuel PA. Surface sampling of spores in dry-deposition aerosols. *Appl. Environ. Microbiol.* 2009; 75:39–44. [PubMed: 18997021]
- Emanuel, P.; Roos, JW.; Niyogi, K. *Sampling for Biological Agents in the Environment*. ASM Press; Washington, D.C.: 2008.
- Estill CF, Baron PA, Beard JK, Hein MJ, Larsen LD, Rose L, Schaefer FW III, Noble-Wang J, Hodges L, Lindquist HD, Deye GJ, Arduino MJ. Recovery efficiency and limit of detection of aerosolized *Bacillus anthracis* Sterne from environmental surface samples. *Appl. Environ. Microbiol.* 2009; 75:4297–4306. [PubMed: 19429546]

- Franco C, Bouri N. Environmental decontamination following a large-scale bioterrorism attack: federal progress and remaining gaps. *Biosecur. Bioterror.* 2010; 8:107–117. [PubMed: 20367575]
- Frawley DA, Samaan MN, Bull RL, Robertson JM, Mateczun AJ, Turnbull PCB. Recovery efficiencies of anthrax spores and ricin from nonporous or nonabsorbent and porous or absorbent surfaces by a variety of sampling methods. *J. Forensic Sci.* 2008; 53:1102–1107. [PubMed: 18637873]
- Gilchrist, MJR. Microbiological culturing of environmental and medical-device surfaces. In: Isenberg, HD., editor. *Clinical Microbiology Procedures Handbook*. American society for Microbiology; Washington, D.C.: 1992.
- Hodges LR, Rose LJ, O'Connell H, Arduino MJ. National validation study of a swab protocol for the recovery of *Bacillus anthracis* spores from surfaces. *J. Microbiol. Methods.* 2010; 81:141–146. [PubMed: 20193714]
- Lee SD, Ryan SP, Snyder EG. Development of an aerosol surface inoculation method for *Bacillus* spores. *Appl. Environ. Microbiol.* 2011; 77:1638–1645. [PubMed: 21193670]
- Lee SD, Calfee MW, Mickelsen L, Wolfe S, Griffin J, Clayton M, Griffin-Gatchalian N, Touati A. Evaluation of surface sampling for *Bacillus* spores using commercially available cleaning robots. *Environ. Sci. Technol.* 2013; 47:2595–2601. [PubMed: 23431954]
- Nakamura LK. Taxonomic relationship of black-pigmented *Bacillus subtilis* strains and a proposal for *Bacillus atrophaeus* sp. nov. *Int. J. Syst. Bacteriol.* 1989; 39:295–300.
- Piepel GF, Amidan BG, Hu R. Laboratory studies on surface sampling of *Bacillus anthracis* contamination: summary, gaps and recommendations. *J. Appl. Microbiol.* 2012; 113:1287–1304. [PubMed: 22747878]
- Rastogi VK, Wallace L, Smith LS, Ryan SP, Martin B. Quantitative method to determine sporicidal decontamination of building surfaces by gaseous fumigants, and issues related to laboratory-scale studies. *Appl. Environ. Microbiol.* 2009; 75:3688–3694. [PubMed: 19346341]
- Rose LJ, Hodges L, O'Connell H, Noble-Wang J. National validation study of a cellulose sponge wipe-processing method for use after sampling *Bacillus anthracis* spores from surfaces. *Appl. Environ. Microbiol.* 2011; 77:8355–8359. [PubMed: 21965403]
- Schmitt K, Zacchia NA. Total decontamination cost of the anthrax letter attacks. *Biosecur. Bioterror.* 2012; 10:98–107. [PubMed: 22313022]
- Sehulster, LM.; Rose, LJ.; Noble-Wang, J. Microbiologic sampling of the environment in healthcare facilities. In: Mayhall, GC., editor. *Hospital Epidemiology and Infection Control*. Lippincott Williams & Wilkins; Philadelphia, PA: 2012.
- Teshale EH, Painter J, Burr GA, Mead P, Wright SV, Cseh LF, Zabrocki R, Collins R, Kelley KA, Hadler JL, Swardlow DL, Team M.O.T.C.A.R. Environmental sampling for spores of *Bacillus anthracis*. *Emerg. Infect. Dis.* 2002; 8:1083–1087. [PubMed: 12396920]
- U.S. Government Accountability Office. Anthrax detection: agencies need to validate sampling activities in order to increase confidence in negative results. Washington, DC: 2005. Report No. GAO-05-251
- Wood JP, Choi YW, Rogers JV, Kelly TJ, Riggs KB, Willenberg ZJ. Efficacy of liquid spray decontaminants for inactivation of *Bacillus anthracis* spores on building and outdoor materials. *J. Appl. Microbiol.* 2011; 110:1262–1273. [PubMed: 21332900]

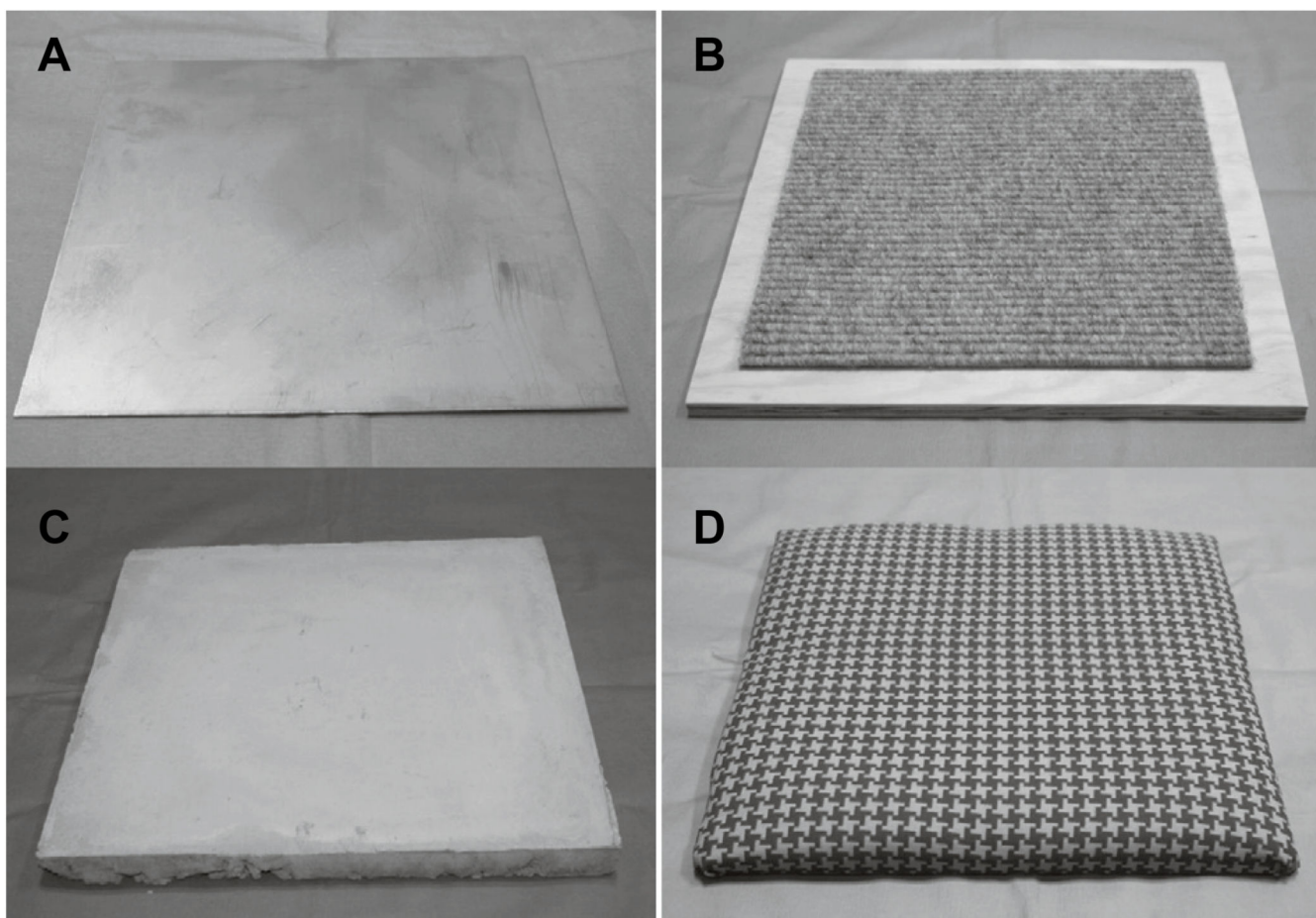


Fig. 1.

Photograph of representative material surface coupons. Stainless steel (A) coupons (35.6 cm by 35.6 cm) were sampled with pre-moistened gauze wipes, and the resulting recoveries were used to normalize vacuum-based recoveries across trials. Coupons (35.6 cm by 35.6 cm) of carpet (B), concrete (C), and upholstery (D) were sampled with vacuum-based methods and represented surface materials common to indoor or outdoor environments.

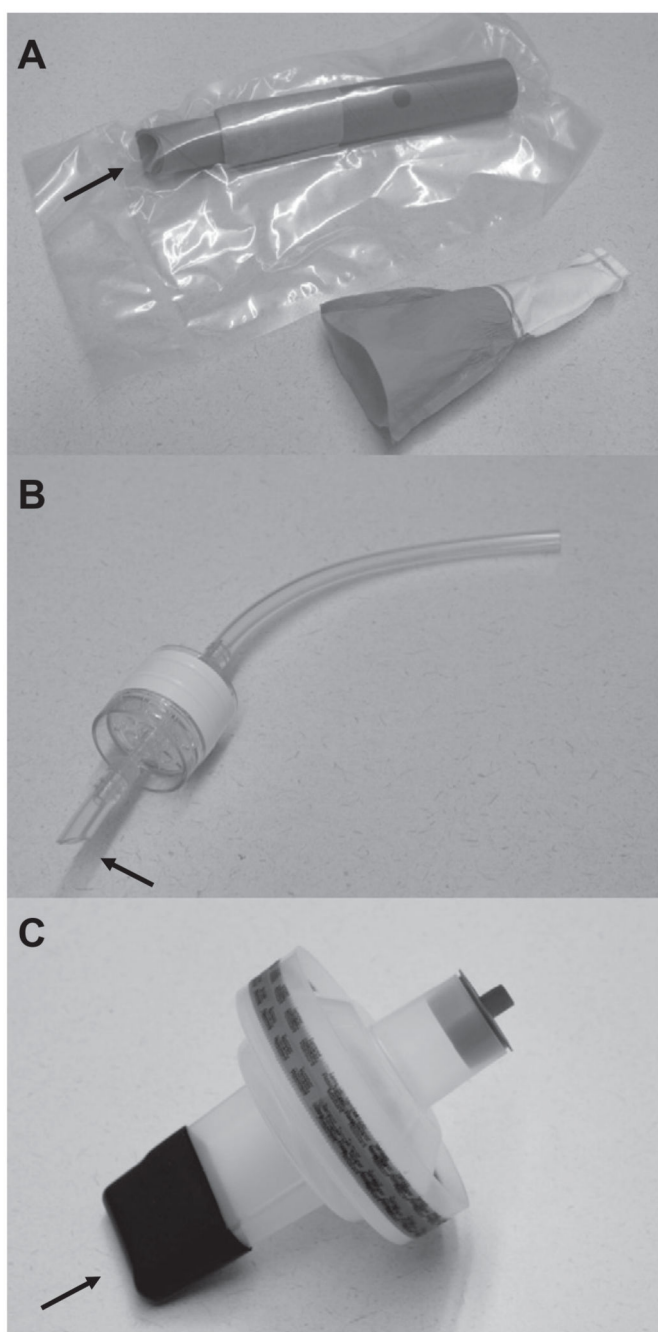


Fig. 2. Photograph of vacuum-based sampling devices. Among currently-used vacuum-based surface sampling devices are the vacuum sock (A), 37 mm cassette filter (B), and forensic filter (C). The inlet of each device is indicated by a black arrow.

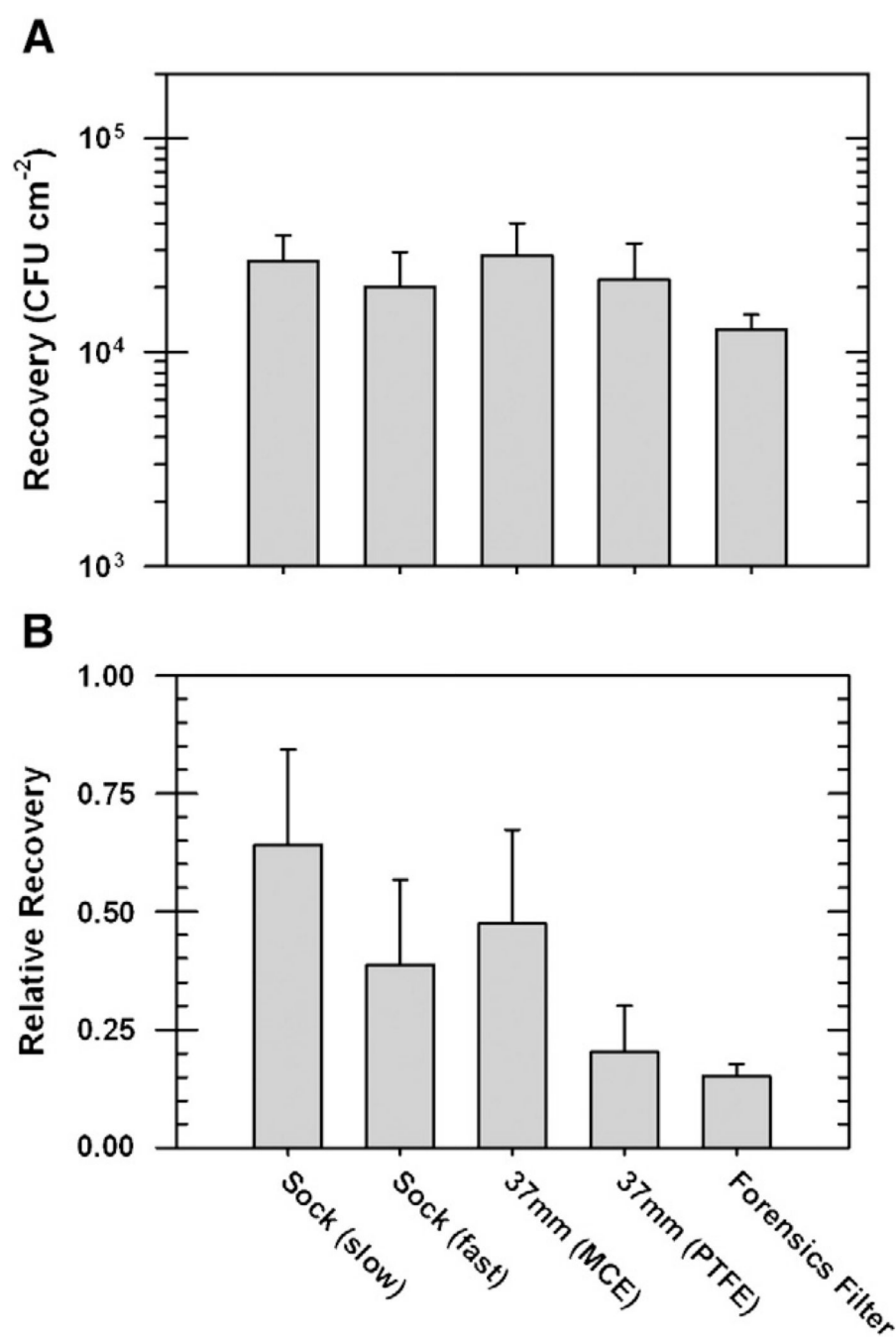


Fig. 3.

Vacuum-based sampling recoveries from carpet. Total recoveries (A) and relative recoveries (B) for the vacuum sock (slow), vacuum sock (fast), 37 mm cassette filter (MCE), 37 mm cassette filter (PTFE), and forensic filter methods, achieved when used to sample inoculated carpet coupons. Relative recoveries were determined by dividing the mean number of spores recovered by each vacuum method by the mean number of spores recovered by wipe sampling stainless steel positive control coupons. All data are presented as the mean \pm one

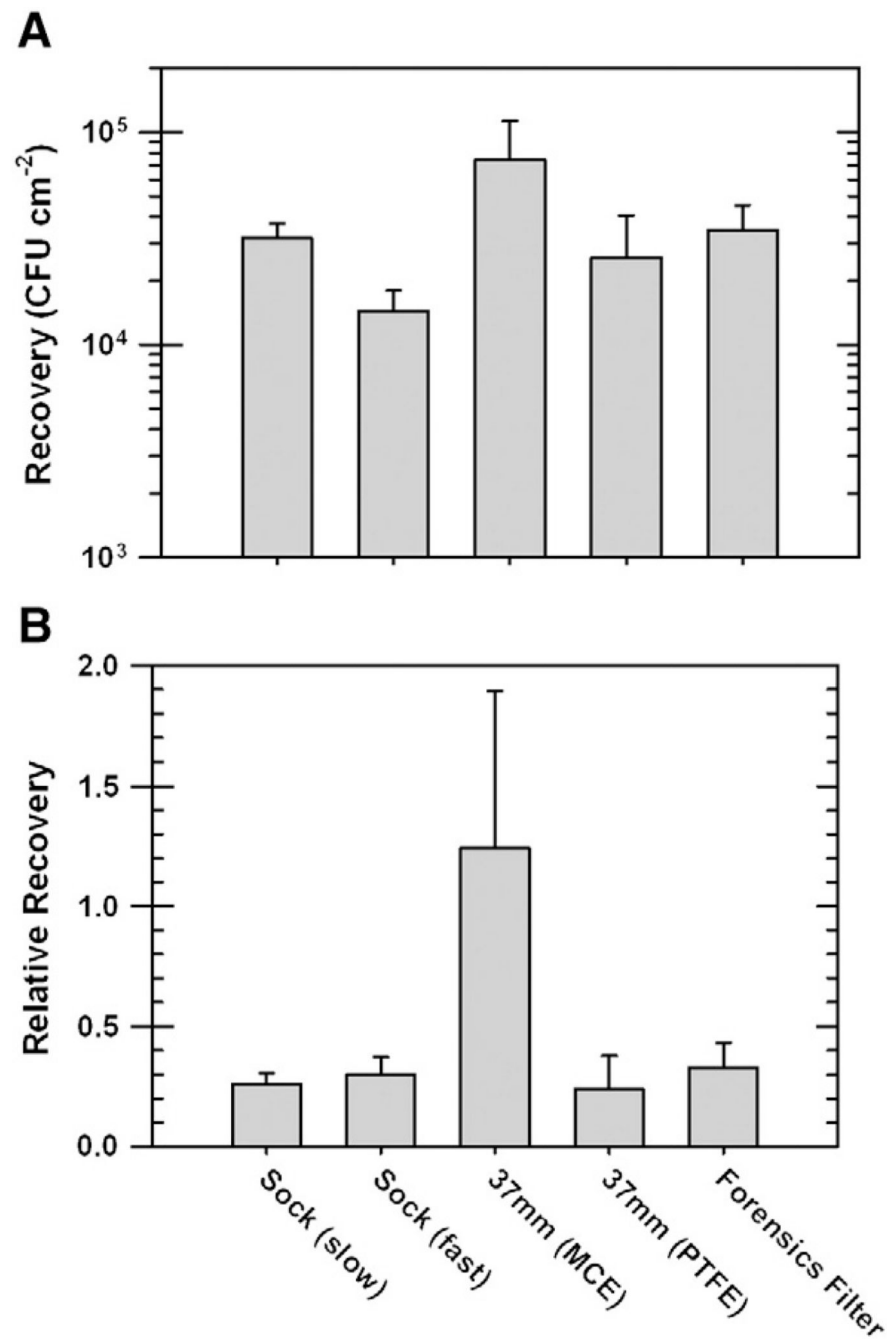
standard deviation from ten replicate test samples, and five replicate positive control samples.

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**Fig. 4.**

Vacuum-based sampling recoveries from concrete. Total recoveries (A) and relative recoveries (B) for the vacuum sock (slow), vacuum sock (fast), 37 mm cassette filter (MCE), 37 mm cassette filter (PTFE), and forensic filter methods, achieved when used to sample inoculated concrete coupons. Relative recoveries were determined by dividing the mean number of spores recovered by each vacuum method by the mean number of spores recovered by wipe sampling stainless steel positive control coupons. All data are presented

as the mean \pm one standard deviation from ten replicate test samples, and five replicate positive control samples.

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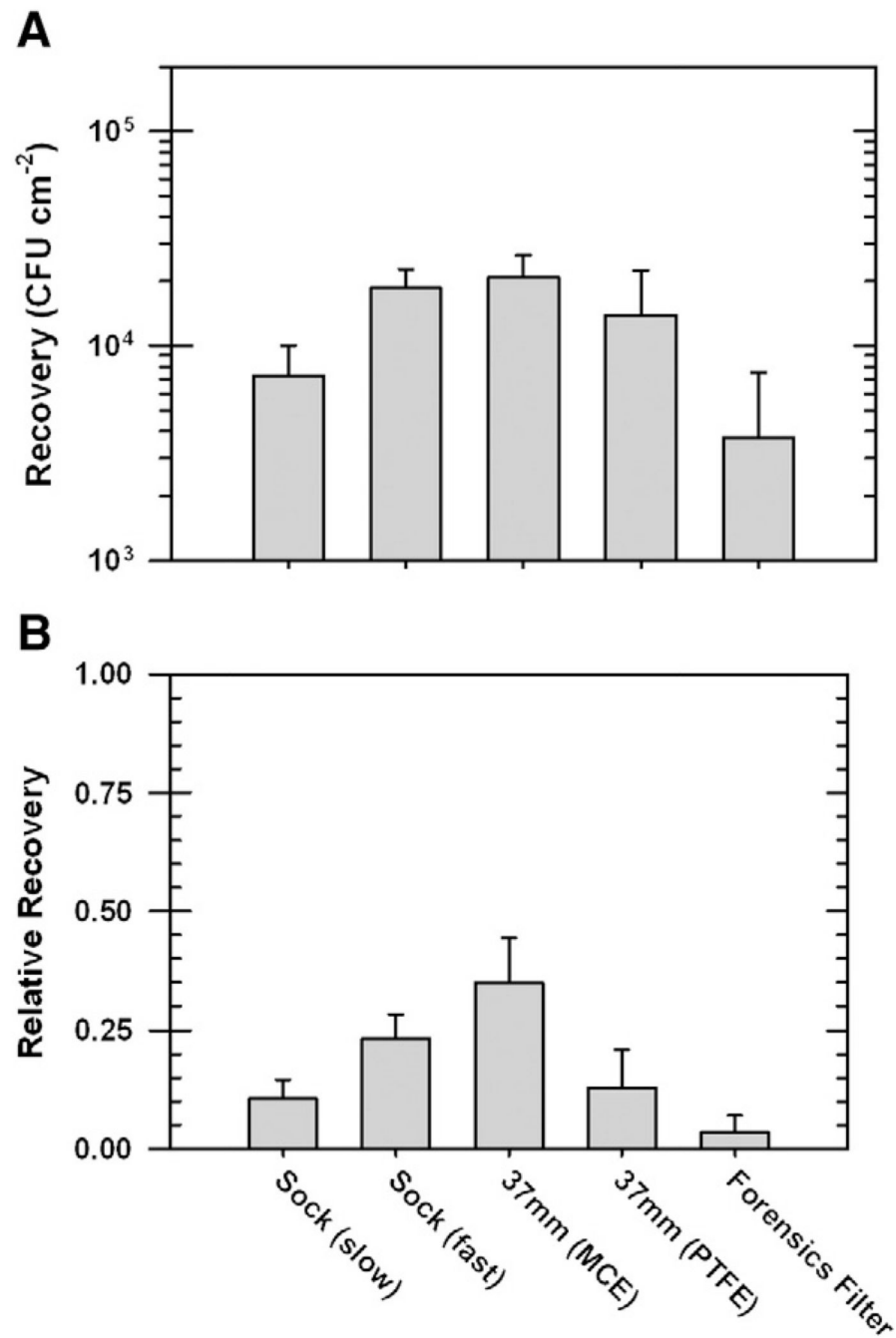


Fig. 5. Vacuum-based sampling recoveries from upholstery. Total recoveries (A) and relative recoveries (B) for the vacuum sock (slow), vacuum sock (fast), 37 mm cassette filter (MCE), 37 mm cassette filter (PTFE), and forensic filter methods, achieved when used to sample inoculated concrete coupons. Relative recoveries were determined by dividing the mean number of spores recovered by each vacuum method by the mean number of spores recovered by wipe sampling stainless steel positive control coupons. All data are presented

as the mean \pm one standard deviation from ten replicate test samples, and five replicate positive control samples.

Table 1

Summary of vacuum-based sampling devices.

| Vacuum-based sampling device | Manufacturer | Part# | Flow rate ^a (L min ⁻¹) |
|--|------------------------------------|------------|--|
| Vacuum sock | Midwest Filtration; Cincinnati, OH | x-cell-200 | 2000 |
| 37 mm MCE cassette, 0.8 µm pore size | SKC, Inc.; Eighty Four, PA | 225-3-01 | 20 |
| 37 mm PTFE cassette, 0.3 µm pore size | SKC, Inc.; Eighty Four, PA | 225-1723 | 20 |
| Forensic filter | 3 M; St. Paul, MN | FF1 | 790 |

^a Air flow rates are specific for the current study, and may vary according to vacuum source used.

Table 2

Experimental design. All coupons were inoculated with $\sim 1 \times 10^5$ CFU cm⁻².

| Trial | Sampling method | Sample area (cm ²) | Surface material | Replicates (n) |
|-------|-------------------------|--------------------------------|------------------|----------------|
| 1 | Vacuum sock (fast) | 2787 | Carpet | 10 |
| | | | Concrete | 10 |
| | | | Upholstery | 10 |
| | Gauze wipe ^a | 929 | Stainless steel | 5 |
| 2 | Vacuum sock (slow) | 2787 | Carpet | 10 |
| | | | Concrete | 10 |
| | | | Upholstery | 10 |
| | Gauze wipe ^a | 929 | Stainless steel | 5 |
| 3 | 37 mm MCE | 929 | Carpet | 10 |
| | | | Concrete | 10 |
| | | | Upholstery | 10 |
| | Gauze wipe | 929 | Stainless steel | 5 |
| 4 | 37 mm PTFE | 929 | Carpet | 10 |
| | | | Concrete | 10 |
| | | | Upholstery | 10 |
| | Gauze wipe | 929 | Stainless steel | 5 |
| 5 | Forensic filter | 2787 | Carpet | 10 |
| | | | Concrete | 10 |
| | | | Upholstery | 10 |
| | Gauze wipe ^a | 929 | Stainless steel | 5 |

^a Three test days (one for each material type) were required for those devices (vacuum sock and forensic filter) where 3 coupons per sample were sampled. Accordingly, a unique set of gauze wipe samples were collected on each test day.

Table 3

Summary of vacuum and wipe sampling recoveries for each trial.

| Material | Trial ^a | Vacuum recovery ($\times 10^6$ CFU cm ⁻²) | | Wipe recovery ($\times 10^3$ CFU cm ⁻²) | | Relative recovery ^b | ANOVA ^c (p value) |
|------------|--------------------|---|-----------|---|-----------|--------------------------------|------------------------------|
| | | Mean | Std. dev. | Mean | Std. dev. | | |
| Carpet | 1 | 20.1 | 9.3 | 51.8 | 13.0 | 0.387 | <0.001 |
| | 2 | 26.6 | 8.5 | 41.6 | 21.6 | 0.641 | |
| | 3 | 28.2 | 11.9 | 59.6 | 12.6 | 0.474 | |
| | 4 | 21.8 | 10.5 | 107.7 | 38.7 | 0.202 | |
| | 5 | 12.8 | 2.2 | 84.2 | 29.0 | 0.151 | |
| Concrete | 1 | 14.4 | 3.6 | 48.1 | 18.9 | 0.299 | <0.001 |
| | 2 | 31.8 | 5.5 | 122.6 | 60.5 | 0.259 | |
| | 3 | 74.1 | 38.8 | 59.6 | 12.6 | 1.242 | |
| | 4 | 25.7 | 14.9 | 107.7 | 38.7 | 0.238 | |
| | 5 | 34.7 | 10.6 | 105.3 | 40.5 | 0.330 | |
| Upholstery | 1 | 18.6 | 4.2 | 80.4 | 32.7 | 0.232 | <0.001 |
| | 2 | 7.3 | 2.7 | 68.7 | 27.4 | 0.106 | |
| | 3 | 20.8 | 5.6 | 59.6 | 12.6 | 0.350 | |
| | 4 | 13.9 | 8.6 | 107.7 | 38.7 | 0.129 | |
| | 5 | 3.8 | 3.8 | 108.0 | 41.5 | 0.035 | |

Bold values indicate trial with the highest recovery, as indicated by Bonferroni post hoc tests, for each material. For carpet trials, no one method demonstrated superior recovery compared to the other methods.

^a Trials are consistent with Table 2, and are as follows: Trial 1, vacuum sock (fast); Trial 2, vacuum sock (slow); Trial 3, 37 mm cassette (MCE); Trial 4, 37 mm cassette (PTFE); Trial 5, forensic filter.

^b Relative recovery = mean vacuum recovery / mean wipe recovery.

^c ANOVA (one-way) conducted on relative recoveries, across trials (methods) yet within each material.